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Detection of Interleukin-1 β in the Tear Fluid of Patients With Corneal Disease With or Without Conjunctival Involvement

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Abstract: To investigate the role of Interleukin-1 (IL-1) in the pathobiology of the cornea, we measured IL-1 β concentration in tear fluid samples from patients with corneal disease. Twenty patients with unilateral corneal disease were included in the study. Tear fluid samples were collected during the active stages of the disease and following resolution. The fellow (unaffected) eyes served as controls. The concentration of IL-1 β in the tear fluid samples was measured using a sandwich ELISA method. IL-1 β was detected in tear fluid from five eyes (three eyes with chemical burns, one with a Pseudomonas aeruginosa corneal ulcer, and one with a peripheral corneal ulcer) at concentrations between 29 and 218 pg/mL. IL-1 β was not detected in tear fluid from the remaining 15 affected eyes, nor from the control eyes. The detection of IL-1 β in tear fluid correlated with limbal conjunctival involvement, but did not correlate with the type of disease, size of epithelial defect, or degree of stromal involvement. IL-1 β in the tear fluid may be one of the factors modifying the complex inflammatory process of the anterior ocular surface. **Jpn J Ophthalmol 1997;41:63–66** © 1997 Japanese Ophthalmological Society

Key Words: Corneal disease, interleukin-1 β, tear fluid.

Introduction

Interleukin-1 (IL-1) is a cytokine that plays an important role in inflammatory responses. The two types of IL-1, namely IL-1 α and IL-1 β, show only about 25% sequence identity but have almost identical functions.[1] Studies of ocular surface tissue indicate that cultured corneal epithelial cells contain IL-1 messenger-RNA,[2] and release IL-1.[3,4] It has been reported that the expression of IL-1 β in the cornea increases during herpetic stromal keratitis,[5] and in the injured vitamin A deficient cornea.[6] IL-1 has also been reported to induce collagenolytic activity by keratocytes on plastic plates[7] and in collagen gel,[8] and to promote corneal neovascularization.[9] However, the presence of IL-1 β in tear fluid, and its dynamic changes during corneal disease and healing, have not been investigated. Therefore, we conducted this study to determine whether IL-1 β can be detected in tear fluid from normal eyes, and from eyes with various types of corneal trauma and disease. IL-1 β was detected in some cases of severe corneal disease, but only during the active stage of the disease process.

Materials and Methods

Subjects

Twenty patients (12 males and eight females) with active unilateral corneal disease or trauma, ranging in age from 18 to 80 years, participated in the study. Informed consent in written form was obtained from all subjects prior to participation. The group of patients included eight cases of chemical burn, six cases of corneal ulceration, three cases of herpetic keratitis, two cases of recurrent erosion, and one case of Mooren's ulcer.

Clinical Evaluation

Prior to tear sample collection during the active stage of the disease process, slit lamp biomicroscopy was used to determine the area of epithelial loss, and the levels of stromal involvement (stromal damage

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associated with the epithelial loss) and limbal conjunctival involvement (conjunctival epithelial defects or severe inflammation accompanied with corneal disorder).

Tear Collection

Tear fluid samples were collected from both eyes using glass microcapillary tubes, as described elsewhere,[10] and frozen immediately at −20°C until analysis. Tear collection was conducted during the active stage of the disease (approximately 30 μL of tears collected) and again following resolution of the disease process (approximately 5 μL collected). The fellow (unaffected) eyes acted as controls (approximately 5 μL collected).

Extreme care was taken to minimize ocular surface contact during tear sample collection. Patients were encouraged to report any excessive irritation or induction of reflex tearing during sample collection. In addition, examination of the ocular surface using slit lamp biomicroscopy and fluorescein was performed on all patients after tear collection to discount the possibility of significant ocular surface damage.

Determination of IL-1 β Concentration

A commercially available sandwich ELISA technique was used to determine the concentration of IL-1 β in the tear samples (Quantikine; R&D Systems, Minneapolis, MN, USA). The lower limit of this assay was 3.9 pg/mL. Briefly, the thawed tear sample volume was measured, then diluted with PBS to a final volume of 50 μL and applied to microplate wells on which a murine monoclonal antibody against IL-1 β had been immobilized. After incubation for 1 hour on a microplate shaker, and three repeat washings with washing buffer, an HRP-conjugated polyclonal antibody against IL-1 β was applied to the microplate wells. After another hour of incubation on a microplate shaker and further washing, the amount of IL-1 β was assayed by a colorimetric method using a microplate reader (Model MTP-120, Corona Electric Co. LTD., Tokyo, Japan), using tetramethylbenzidine/H_2O_2 as the enzyme substrate. IL-1 β concentration in the tear sample was calculated by the dilution factor.

Results

As summarized in Table 1, IL-1 β was detected in tear fluid samples from five eyes (two eyes with al-

Table 1. Concentration of IL-1 β in Tear Samples Collected During Corneal Disease

Case	Age	Sex	Diagnosis	IL-1 β (Active) pg/mL	IL-1 β (Healed) pg/mL	Epithelial Defect Size in Total Corneal Area	Stromal Involvement[a]	Conjunctival Involvement[b]
1	54	M	Alkali burn	151	ND	> .75	yes	yes
2	46	M	Alkali burn	34	ND	.5 to .75	yes	yes
3	27	M	Alkali burn	ND	ND	< .25	yes	no
4	21	M	Alkali burn	ND	ND	.5 to .75	yes	no
5	18	M	Chemical burn	ND	ND	.5 to .75	yes	no
6	69	F	Chemical burn	ND	ND	.25 to .5	yes	no
7	73	M	Chemical burn	35	ND	< .25	no	no
8	69	F	Chemical burn	ND	ND	< .25	no	no
9	52	F	Central ulcer	ND	ND	< .25	yes	no
10	22	F	Peripheral ulcer	ND	ND	< .25	yes	no
11	75	F	Peripheral ulcer	218	ND	< .25	yes	yes
12	20	F	Pseudomonas ulcer	ND	ND	< .25	yes	no
13	80	F	Pseudomonas ulcer	29	ND	< .25	yes	yes
14	45	M	Fungal ulcer	ND	ND	< .25	yes	no
15	31	M	Epithelial herpetic keratitis	ND	ND	< .25	yes	no
16	50	M	Stromal herpetic keratitis	ND	ND	< .25	yes	no
17	43	M	Meta-herpes	ND	ND	< .25	yes	no
18	43	M	Recurrent erosion	ND	ND	< .25	no	no
19	58	M	Recurrent erosion	ND	ND	< .25	no	no
20	79	F	Mooren's ulcer	ND	ND	< .25	yes	yes

ND: not detectable (< 3.9 pg/mL in diluted tear sample).
[a]Stromal involvement—corneal stromal damage associated with the epithelial disorder.
[b]Conjunctival involvement—conjunctival damage associated with the corneal disorder.

kali burns, one eye with other chemical burns, one eye with a corneal ulcer due to Pseudomonas aeruginosa, and one eye with a peripheral corneal ulcer). The concentration of IL-1 β in these five cases ranged from 29 to 218 pg/mL. However, following healing, the level of IL-1 β in these cases had dropped below detectable levels (< 3.9 pg/mL in diluted tear sample). IL-1 β was not detected in tear fluid samples obtained from the other 15 cases of corneal disease, nor from any of the control eyes. In four out of five cases in which IL-1 β was detected in tear fluid samples, there was conjunctival involvement in the corneal disease process. However, there was no correlation between detection of IL-1 β and the type of disease, size of epithelial defect, or level of stromal involvement.

Two cases that revealed high levels of IL-1 β in the tear fluid warrant some brief discussion. Case 1 was a 54-year-old male who had sustained an extensive alkali burn in the left eye, involving both cornea and conjunctiva. The area of epithelial loss involved the entire cornea, except for the nasal and superior limbus, and extended to include the inferior and temporal conjunctiva (Figure 1). The concentration of IL-1 β in the tear fluid in this eye was 151 pg/mL. The injury was treated with topical antibiotics, steroids, and fibronectin eye drops. Following initial healing, recurrence of the epithelial defect was observed only once during the first month. Final visual acuity was 20/20 (6/6). Case 11 was a 75-year-old female who presented at our clinic with a superior nasal peripheral culture negative corneal ulcer in the left eye, with severe conjunctival hyperemia and edema adjacent to the ulcer (Figure 2). The concentration of IL-1 β in the tear fluid sample from this patient was 218 pg/mL. The ulcer did not respond to

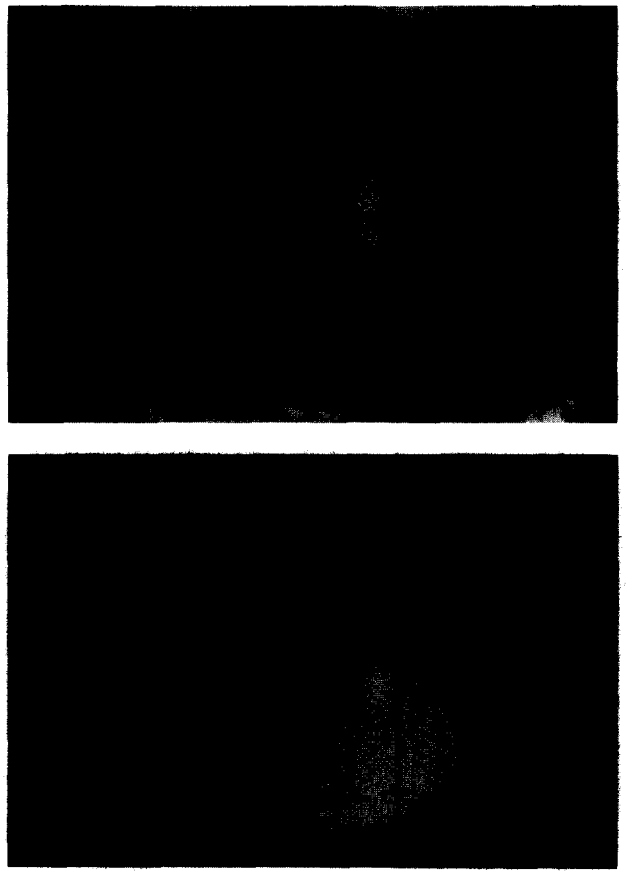

Figure 1. Slit lamp photographs of the affected (left) eye in Case 1 (chemical burns). Note the opaque area in the lower temporal corneal quadant (**A**). The corneal epithelial defect covers over 3/4 of the total corneal area, and is accompanied by temporal and inferior bulbar conjunctival epithelial defects (**B**). Corneal epithelium at the nasal and superior limbus is intact (**B**).

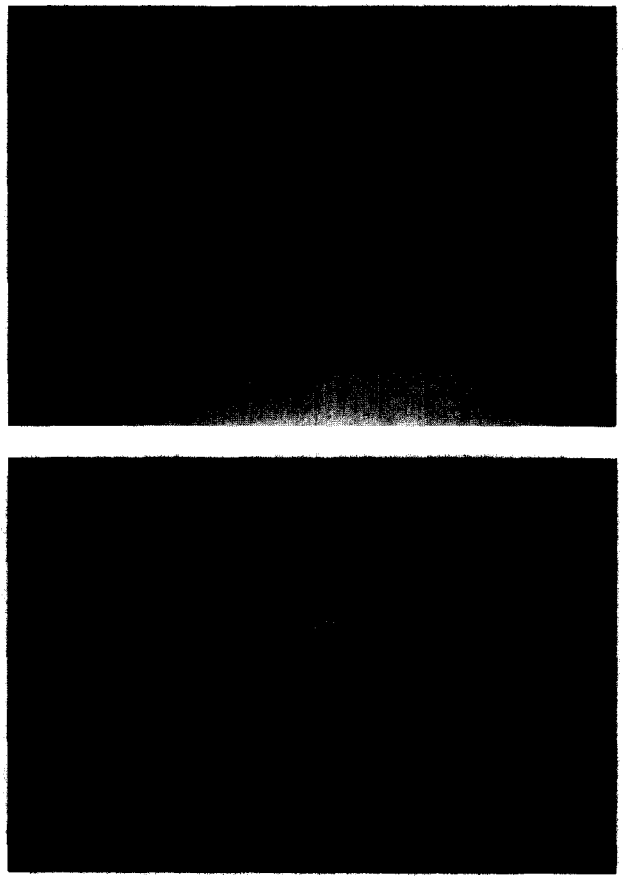

Figure 2. Slit lamp photographs of the affected (left) eye in Case 11 (peripheral corneal ulcer). Note the severe stromal infiltration associated with the peripheral ulcer in the superior nasal cornea, and the severe conjunctival hyperemia and edema adjacent to the ulcer (**A**). A localized peripheral corneal epithelial defect was clearly observed after instillation of fluorescein (**B**).

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topical or systemic antibiotic therapy, but eventually healed with the application of steroid eye drops.

Discussion

In this study, we have demonstrated that IL-1 β can be detected in the tear fluid in some corneal diseases and injuries. Cases 1 and 11, especially with over 100 pg/mL of IL-1 β in the tear fluid, had persistent corneal epithelial defects as well as severe corneal involvement. Because we detected IL-1 β only during the active stage of the disease process, we believe that IL-1 β is not a usual or basic component of human tear fluid in the normal eye, but is actively released from ocular surface tissue during inflammation.

During corneal disease, leukocytes migrate from dilated conjunctival blood vessels and participate in the process of inflammation. Thus, possible sources for tear fluid IL-1 β in the cases of corneal disease reported here include migrating leukocytes and the stimulated or traumatized ocular surface tissue (cornea and/or conjunctiva). The correlation found in this study between detection of IL-1 β in tear fluid and conjunctival involvement suggests that the inflamed and hyperemic conjunctival tissue may be a major source for this cytokine. Further investigation is warranted to determine the precise source of IL-1 β in tear fluid during corneal disease and trauma.

IL-1 β in the tear fluid may act to promote ocular surface inflammation in a number of ways. For example, the concentrations of other interleukins, such as IL-6, which plays a role in cell proliferation and differentiation, and IL-8, which is a chemoattractant for neutrophils and lymphocytes, have been shown to increase in association with increased concentrations of IL-1. In the murine herpetic keratitis model, both IL-1 and IL-6 concentrations are elevated in the cornea, with the former peaking 6 hours before the latter. In in vitro studies, the presence of IL-1 has been shown to induce a significant increase in IL-8 gene expression in corneal epithelial cells, keratocytes, and endothelial cells.[11,12] IL-1 has also been shown to stimulate thromboxane production by corneal epithelial cells,[13] and collagenase production by keratocytes.[7,8] It is suggested that IL-1 β in the tear fluid might promote corneal epithelial wound healing. The IL-6 induction, however, might inhibit it by the recruitment of PMNs and production of thromboxane and collagenase in corneal tissue. The relationship between the cytokine network and the process of ocular surface inflammation requires further investigation to clarify the role of these substances in ocular inflammation.

In summary, we have detected IL-1 β in the tear fluid of patients with active corneal disease. Measurement of IL-1 β concentrations in tear fluid may be of clinical importance because of the role of IL-1 β in the initiation of ocular surface inflammation.

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